DETAILED ACTION

Response to Amendment

1. Applicant's response to the non-compliant amendment filed on February 28, 2008 and to the office communication filed on July 17, 2008 have been entered. The claims pending in this application are claims 1-16, 21-36, and 39-47 wherein claims 14 and 22 have been withdrawn for the examination due to species election requirement mailed on March 13, 2006. Rejection and /or objection not reiterated from the previous office action are hereby withdrawn in view of applicant's amendment filed on July 17, 2008. Since applicant has elected species (1) (the cells comprise bacterioplankton, see claim 43) in the response filed on November 2, 2007, claims 1-13, 15, 16, 21, 23-36 and 39-47 will be examined.

Drawings

2. Newly submitted Figures 26A to 26D filed on June 6, 2007 have been accepted by the office.

Claim Objections

3. Claim 1 or 39 or 40 is objected to because of the following informality: "the culture medium" in step (g) should be "the culture media" in order to correspond to step (b).

4. Claim 4 is objected to because of the following informality: "a eukaryotic cell" should be "an eukaryotic cell".

5. Claim 11 is objected to because of the following informality: "an extremophile hyperthermophile cell" should be "a hyperthermophile cell".

6. Claim 21 is objected to because of the following informality: "conditions allowing the encapsulated cell to survive and be maintained" should be "the conditions allowing the encapsulated cell to survive and be maintained".

7. Claim 29 is objected to because of the following informality: "sorting the encapsulated microcolony by size" should be "said sorting the encapsulated microcolony by size".

8. Claim 44 or 46 or 47 is objected to because of the following informality: "a filter membrane at the inlet and outlet" should be "filter membranes at the inlet and outlet".

Appropriate correction is required.

Claim Rejections - 35 USC § 112

9. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

10. New Matter

Claim 1-13, 15, 16, 21, 23-36 and 39-47 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The limitation "a growth column with an inlet and an outlet" is added to the newly amended independent claims 1, 39, and 40, newly added claim 44 contains the limitation "the growth column further comprises a filter membrane at the inlet and the outlet", newly added claim 46 contains the limitation "the growth column further comprises a filter membrane at the

inlet and the outlet, and the filters prevent free-living cells from contaminating the media reservoir", and newly added claim 47 contains the limitation "the growth column further comprises a filter membrane at the inlet and the outlet, and the filters retain the porous microdroplets in the growth column while allowing non-encapsulated cells to be washed out of the growth column by the flow of the growth medium". Although the specification describes that "[T]he GMDs were dispensed into sterile chromatography columns XK-16 (Pharmacia Biotec) containing 25 ml of media. Columns were equipped with two sets of filter membranes (0.1 μm at the inlet of the column and 8 μm at the outlet). The filters prevented free-living cells contaminating the media reservoir and retained GMDs in the column while allowing free-living cells to be washed out" (see page 168, last paragraph bridging to page 169, first paragraph), the specification fails to define or provide any disclosure to support these limitations recited in claims 1, 39, 40, 44, 46, and 47 since not all growth columns in the specification have an inlet and an outlet and the growth columns having an inlet and an outlet in the specification are sterile chromatography columns XK-16. Newly added claim 41 contains a limitation "the growth media comprises an amino acid supplemented medium; an organic rich medium diluted in seawater; a marine medium; a seawater amended with a mixture of amino acids; a seawater amended with inorganic nutrients; a sterile filtered seawater; a diluted soil extract, or a combination thereof". Although the specification describes that the growth media comprises an amino acid supplemented medium; an organic rich medium diluted in seawater; a marine medium; a seawater amended with a mixture of amino acids; a seawater amended with inorganic nutrients; a sterile filtered seawater; a diluted soil extract (see the specification, page 164, second paragraph, and page 166, third paragraph), the specification fails to define or provide any disclosure to

support that "the growth media comprises a combination of an amino acid supplemented medium, an organic rich medium diluted in seawater, a marine medium, a seawater amended with a mixture of amino acids, a seawater amended with inorganic nutrients, a sterile filtered seawater, and a diluted soil extract" recited in claim 41. Newly added claim 42 contains a limitation "wherein the encapsulated cells are incubated in the porous microdroplet in the growth column for up to five weeks, or at least for between three hours and five weeks, or from about between 20 minutes and several weeks or months". Although the specification describes that "10 *Escherichia coli* cells (expressing a green fluorescent protein, ZsGreen, Clontech) are individually encapsulated and incubated for three hours to form microcolonies within the GMDs" (see page 169, third paragraph), "after five weeks of incubation, 1200 GMDs, each containing a microcolony, are collected by flow cytometry from each of the four growth columns" (page 164, second paragraph), and "over a period of time (20 minutes to several weeks or months), a clonal population of the preferably one organism grows within the microenvironment" (see page 39, third paragraph), the specification does not describe that the encapsulated cells are incubated in the porous microdroplet in the growth column for up to five week or at least between three hours and five weeks or about 20 minutes to several weeks or months as recited in claim 42 because up to five week includes 3 week and at least between three hours and five weeks includes 5 hour which are not described in the specification and the phrase "from about 20 minutes to several weeks or months" recited in claim 42 is much broader than the phrase "20 minutes to several weeks or months". Newly added claim 43 contains a limitation "wherein the cells comprise bacterioplankton, *Planctomycetales*, *Planctomycetes*, or *Cytophaga*, or *Lavobacterium*, or *Bacteroides*, or *Proteobacteria*, or *Salinibacter*, or *Rhodothermus*, or

Methanococcus", nowhere in the specification describes *Lavobacterium* and describes that the cells comprise bacterioplankton, *Planctomycetales*, *Planctomycetes*, or *Cytophaga*, or *Lavobacterium*, or *Bacteroides*, or *Proteobacteria*, or *Salinibacter*, or *Rhodothermus*, or *Methanococcus* together as recited in claim 43.

MPEP 2163.06 notes "IF NEW MATTER IS ADDED TO THE CLAIMS, THE EXAMINER SHOULD REJECT THE CLAIMS UNDER 35 U.S.C. 112, FIRST PARAGRAPH - WRITTEN DESCRIPTION REQUIREMENT. *IN RE RASMUSSEN*, 650 F.2D 1212, 211 USPQ 323 (CCPA 1981)." MPEP 2163.02 teaches that "Whenever the issue arises, the fundamental factual inquiry is whether a claim defines an invention that is clearly conveyed to those skilled in the art at the time the application was filed...If a claim is amended to include subject matter, limitations, or terminology not present in the application as filed, involving a departure from, addition to, or deletion from the disclosure of the application as filed, the examiner should conclude that the claimed subject matter is not described in that application." MPEP 2163.06 further notes "WHEN AN AMENDMENT IS FILED IN REPLY TO AN OBJECTION OR REJECTION BASED ON 35 U.S.C. 112, FIRST PARAGRAPH, A STUDY OF THE ENTIRE APPLICATION IS OFTEN NECESSARY TO DETERMINE WHETHER OR NOT "NEW MATTER" IS INVOLVED. *APPLICANT SHOULD THEREFORE SPECIFICALLY POINT OUT THE SUPPORT FOR ANY AMENDMENTS MADE TO THE DISCLOSURE*" (emphasis added).

11. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

12. Claims 1-13, 15, 16, 21, 23-36 and 39-47 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

13. Claim 1 or 39 or 40 recites the limitation "the culture media" in step (a) of the claim. There is insufficient antecedent basis for this limitation in the claim because there is no phrase "culture media" before "the culture media" in step (a). Please clarify.

14. Claim 1 or 2 or 5 or 39 or 40 is rejected as vague and indefinite in view of step (d) of claim 1 or 39 or 40 because, if cells are uncultivated, it is unclear why the cells can be isolated and/or maintained. Please clarify.

15. Claim 1 or 39 or 40 is rejected as vague and indefinite in view of steps (a) to (f). Since steps (a) to (e) do not produce a porous microdroplet comprising the encapsulated cell, it is unclear why encapsulating in the encapsulation composition at least a single cell from the mixed population of uncultivated cells can create a microenvironment in the porous microdroplet as recited in step (e) and why the porous microdroplet comprising the encapsulated cell can be placed in the growth column as recited in step (f). Please clarify.

16. Claim 1 or 39 or 40 is rejected as vague and indefinite in view of step (g) because it is unclear why incubation conditions can comprise a method step, flowing the culture medium through the length of the growth column. Please clarify.

17. Claim 1 or 39 or 40 recites the limitation "the encapsulated cell" in step (f) of the claim. There is insufficient antecedent basis for this limitation in the claim because there is no phrase "encapsulated cell" in steps (a) to (e). Please clarify.

18. Claim 8 recites the limitation "the encapsidated cell" in the claim. There is insufficient antecedent basis for this limitation in the claim because claim 1 only contains "the encapsulated cell". Please clarify.

19. Claim 12 is rejected as vague and indefinite because it is unclear that a single cell in the claim is from at least a single cell from the mixed population of uncultivated cells or not. Please clarify.

20. Claim 15 is rejected as vague and indefinite because it is unclear that one cell in the claim is from at least a single cell from the mixed population of uncultivated cells or not. Furthermore, since there is no porous gel microdroplet in claim 1, it is unclear why one cell can be encapsulated in each porous gel microdroplet (GMD). Please clarify.

21. Claim 16 is rejected as vague and indefinite because it is unclear that one to four cells in the claim is/are from at least a single cell from the mixed population of uncultivated cells or not. Furthermore, since there is no porous gel microdroplet in claim 1, it is unclear why one to four cells can be encapsulated in each porous gel microdroplet (GMD). Please clarify.

22. Claim 21 is rejected as vague and indefinite because it is unclear why incubation conditions can comprise a method step, providing nutrients at *in situ* concentrations. Please clarify.

23. Claim 23 is rejected as vague and indefinite because it is unclear whether at least two daughter cell is from the encapsulated cell or not. Please clarify.

24. Claim 26 is rejected as vague and indefinite. Since claims 1 and 23 do not indicate that the gel microdroplet comprises a microcolony, it is unclear why a microcolony can be isolated from the gel microdroplet. Please clarify.

25. Claim 27 is rejected as vague and indefinite. Since claim 26 does not indicate that an isolated microdroplet is obtained by isolating a microcolony from the gel microdroplet, it is unclear why a cell can be isolated from the isolated microdroplet. Please clarify.

26. Claim 28 recites the limitation "the encapsulated microcolony" in the claim. There is insufficient antecedent basis for this limitation in the claim because there is no phrase "encapsulated microcolony" in claims 1, 25, and 26. Please clarify.

27. Claim 36 recites the limitation "the cultured encapsulated cells" in the claim. There is insufficient antecedent basis for this limitation in the claim because there is no phrase "cultured encapsulated cells" in claims 1 and 23. Please clarify.

28. Claim 42 is rejected as vague and indefinite. Since the phrase "at least" has no up limit while the word "between" has both high and low limits, the phrase "at least for between three hours and five weeks" does not make sense. Since the word "between" has both high and low limits and the word "about" has no high and low limits, the phrase "from about between 20 minutes and several weeks or months" does not make sense. Please clarify.

Conclusion

29. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

30. No claim is allowed.

31. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center. The faxing of such papers must conform with the notices published in the Official Gazette, 1096 OG 30

(November 15, 1988), 1156 OG 61 (November 16, 1993), and 1157 OG 94 (December 28, 1993)(See 37 CAR § 1.6(d)). The CM Fax Center number is (571)273-8300.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Frank Lu, Ph.D., whose telephone number is (571)272-0746. The examiner can normally be reached on Monday-Friday from 9 A.M. to 5 P.M.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla, can be reached on (571)272-0735.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

/Frank W Lu /
Primary Examiner, Art Unit 1634
October 23, 2008